

Modifications in Call Characteristics and Sonic Apparatus Morphology During Puberty in *Ophidion rochei* (Actinopterygii: Ophidiidae)

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ABSTRACT Juveniles, females, and males of *Ophidion rochei* share similar external morphology, probably because they are mainly active in the dark, which reduces the role of visual cues. Their internal sonic apparatuses, however, are complex: three pairs of sonic muscles, and highly modified vertebrae and ribs are involved in sound production. The sonic apparatus of males differs from juveniles and females in having larger swimbladder plates (modified ribs associate with the swimbladder wall) and sonic muscles, a modified swimbladder shape and a mineralized structure called the “rocker bone” in front of the swimbladder. All of these male traits appear at the onset of sexual maturation. This article investigates the relationship between morphology and sounds in male *O. rochei* of different sizes. Despite their small size range total length (133–170 mm TL), the five specimens showed pronounced differences in sound-production apparatus morphology, especially in terms of swimbladder shape and rocker bone development. This observation was reinforced by the positive allometry measured for the rocker bone and the internal tube of the swimbladder. The differences in morphology were related to marked differences in sound characteristics (especially frequency and pulse duration). These results suggest that male calls carry information about the degree of maturity. Deprived of most visual cues, ophidiids probably have invested in other mechanisms to recognize and distinguish among individual conspecifics and between ophidiid species. As a result, their phenotypes are externally similar but internally very different. In these taxa, the great variability of the sound production apparatus means this complex system is a main target of environmental constraints. *J. Morphol.* 000:000–000, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: acoustic; allometry; communication; fish; morphology; Ophidiiformes

INTRODUCTION

In *Ophidion rochei*, external features of males and females appear identical (Fig. 1). Moreover, this species has an external phenotype that is very

similar to its sympatric species *Ophidion barbatum* (Fig. 1); they have comparable size, shape, and color (see: Casadevall et al., 1996). Both species live in the Mediterranean Sea (Matallanas and Casadevall, 1999), feed mainly on small crustaceans (Matallanas, 1982), are sand dwelling by day and active at night (Fischer et al., 1987; Jardas, 1996; Matallanas and Casadevall, 1999; Nielsen et al., 1999; Dulčić, 2001; Dulčić et al., 2002). This way of life reduces the importance of vision and may explain the great external phenotypic resemblance between species and sexes of the same species. Nevertheless, environmental constraints are expected to impact at least some of the species-specific characteristics used for conspecific recognition during reproduction, agonistic behavior, or both. Anatomically, the main interspecific and intersexual differences are found in the sound production apparatus (Casadevall et al.,

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1996; Parmentier et al., 2006a; Parmentier et al., 2010a).

The sonic apparatus of *Ophidion rochei* is based on three pairs of extrinsic sonic muscles acting directly or indirectly on the swimbladder (Parmentier et al., 2010a; Kéver et al., 2012). The dorsal sonic muscle inserts on the first neural arch, which is referred to as the neural rocker because it is modified to pivot toward the cranium (Parmentier et al., 2010a). The intermediate sonic muscle inserts directly on the first pair of epineurals which are attached to the neural rocker and connected by ligaments to the swimbladder. In juveniles, the development of the sonic apparatus of both sexes follows a similar ontogenetic trajectory (Casadevall et al., 1996; Kéver et al., 2012). However, after sexual maturation, the sound production apparatus of males differs from females in many respects: males have larger sonic muscles, swimbladder plates (modified third pair of epineurals), and neural rocker, and only males have a highly modified swimbladder (neck, internal tube, and gelatinous substance) associate with a mineralized structure called the rocker bone (Casadevall et al., 1996; Parmentier et al., 2010a; Kéver et al., 2012). The ventral sonic muscles that insert on swimbladder wall in juveniles and females insert on the rocker bone in males (Kéver et al., 2012). Consequently, this structure has a role in male sound production. To our knowledge, the appearance and growth of similar structures related to the sonic apparatus during male sexual maturation has never been studied.

In stable environmental conditions (e.g., over a narrow temperature range and within the same season), intraspecific differences observed for fish sounds have generally been related to fish size (Myrberg et al., 1993; Lobel and Mann, 1995; Crawford et al., 1997; Fine et al., 1999; Amorim et al., 2003; Parmentier et al., 2006b; Colley et al., 2009; Tellechea et al., 2010; Tellechea et al., 2011; Boyle and Tricas, 2011; Bertucci et al., 2012) or dimorphism in the sound producing mechanism (Fine et al., 1990; Brantley et al., 1993; Connaughton, 1997; Lagardère et al., 2005; Tellechea and Norbis, 2012). Although sonic apparatus sexual dimorphism has been described in many fish species, there are only a few descriptions of sex-specific differences in sound characteristics (Ladich, 2007). Sexual dimorphism in sonic apparatus morphology and related sounds were described in Batrachoididae (Fine et al., 1990; Brantley et al., 1993), Sciaenidae (Hill et al., 1987), and Osphronemidae (Kratovich, 1980). In *Porichthys notatus*, for example, only Type I males produce the “humming” advertisement calls (Brantley et al., 1993). This morphotype has sonic muscles that are six fold larger (scaled to body size) than in females and Type II males (Brantley et al., 1993). Recently, Kéver et al. (2012) showed

that sexual dimorphism in *O. rochei* was associated with completely distinct sound types. Males produce pulsed sounds that generally last several seconds while females produce much shorter (generally less than 50 ms) and more tonal sounds.

Behavioral observations indicate that fishes can respond selectively to acoustic stimuli that vary in temporal patterns and frequency content (Myrberg et al., 1978; Spanier, 1979; Vasconcelos et al., 2011). Thus, sound variability plays a role in the social life of some species by providing information for size assessment of the calling individual [e.g., dominant frequency (Lobel and Mann, 1995; Connaughton et al., 2000; Colley et al., 2009)], to identify motivation for mating [e.g., calling rate (Mann and Lobel, 1995; Vasconcelos et al., 2011)] and to recognize conspecifics from other closely related vocal species (e.g., Malavasi et al., 2008; Parmentier et al., 2009). In *O. rochei*, the behaviors associated with sound production are unclear. However, Mann et al. (1997) recorded sounds of male *O. marginatum* before and during mating. Because these sounds are similar to those of male *O. rochei*, they may be produced in a similar context.

We hypothesized that the complex sonic apparatus morphology of male *O. rochei* is necessary for the production of long and specific sounds (e.g., unique pattern in pulse period). These sound characteristics increase the likelihood for a caller to be detected and located by a conspecific. In addition, because of ontogenetic changes in sonic apparatus morphology during sexual maturation (Kéver et al., 2012), sounds may also carry information on caller maturity.

The aim of this study is to investigate the relationship between sound production apparatus modifications and sound characteristics in males.

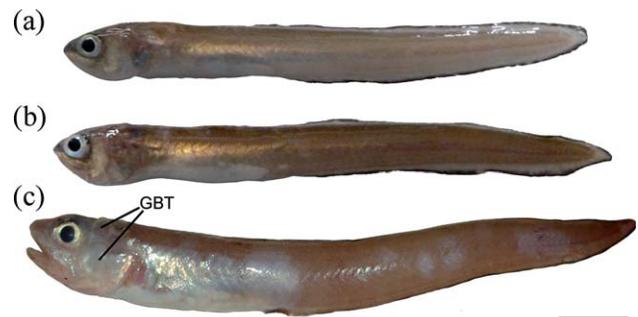


Fig. 1. External phenotypes of a female *Ophidion rochei*, a male *O. rochei*, and an *O. barbatum* individual. Pictures of (a) a female *O. rochei* (150 mm TL), (b) a male *O. rochei* (160 mm TL), and (c) an *O. barbatum* (175 mm TL, sex undetermined). Gas bubble traumas (GBT) caused by pressure variation related to depth change during fish capture (trawling). Scale bar: 20 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

MATERIAL AND METHODS

Fish Collection

Four *Ophidion rochei* Müller, 1845 were caught during July 2010 near the Cetina estuary in Duće-Glava, Croatia (43°26 N, 16°40 E). They were trapped with a beach seine (22 m long, mesh size of 4 mm at the outer wing, and 2 mm at the central part) in shallow water (<2 m depth) from 21:00 to 02:00. They were held for one week in a 250-l tank at the Institute of Oceanography and Fisheries in Split (Croatia). In August 2010, an additional specimen was caught with a small net while scuba diving (10 m depth) in Banyuls-sur-mer, France (42°28 N, 3°08 E). All individuals were transported to Liège (Belgium) and kept in a 1000-l tank with a 0.1 m high sandy bottom. During winter and spring, the temperature and the light-dark cycle were modified to best mimic natural conditions. During the experiment (June to August 2011) temperature was maintained at 21.5°C with 15L:9D cycle.

Sound Recordings

Experimental setup. The study on interindividual differences in male calls was conducted with five captive males. Their calls were recorded from June 24, 2011 to August 2, 2011 at 21.5°C. For each recording, the tank housed a single male with two to three females. The male was separated from the females by a net that enclosed a volume of approximately 10–15 l in the middle of the tank. The hydrophone was always placed at the same spot (half of the water depth, approximately 1 cm from the net). This experimental design limited the distance between the sound producer and hydrophone and thus reduced artifacts caused by reverberation and resonance effects of tank walls (Akamatsu et al., 2002). Each male was placed in the tank for 2 days. This allowed each specimen to be recorded every 10 days. When males were not recorded they were placed in a holding tank at the same temperature as the experimental tank. Female sounds were also recorded but not investigated for individual differences because there were several specimens in the tank and they were free to move relatively far away from the hydrophone.

Recording device and sound analyses. Sounds were recorded with a Digital Spectrogram Long-Term Acoustic Recorder (DSG, Loggerhead Instruments, Sarasota, FL). This device is composed of a hydrophone (–186 dB re 1V/μPa) coupled to a digital acquisition board. It was programmed to record 10 min per half hour during night time at a sample rate of 20 kHz. Sounds were analyzed in Avisoft SAS-Lab Pro 4.5 using the semiautomatic method described in Kéver et al. (2012). Male sounds show specific characteristics: 1) the pulse period rises through the first several pulses, then it alternates between long and short periods in successive pulses (see: Parmentier et al., 2010a; Kéver et al., 2012) and 2) sound spectra were dominated by only two peaks (no harmonics). Thus, the following variables were collected from sounds: pulse number (PN), pulse duration (PD), alternation start (AS) (the PN when the pulse period alternation pattern was initiated), short pulse period (SP) (SPs after the pulse period alternation pattern was initiated), long pulse period (LP) (LPs after the pulse period alternation pattern was initiated), pulse amplitude (A) (maximal amplitude of the pulse in the RMS signal), and the first two peak frequencies (Fig. 2). Each sound contains multiple PDs, As, SPs, and LPs. Thus, each sound includes multiple measurements for these variables. To obtain one value for each variable per sound, the values for each of these variables were measured individually and averaged for each sound. The call duration was not investigated in this study because it is a combination of several other variables (PN, AS, and short and LP).

Morphological Data

All fish investigated in this study had a total length (TL) greater than 130 mm and showed at least an early developing rocker bone (Table 1). According to the classification described

in Kéver et al. (2012), there were both immature and mature males in the studied group. The smallest male found by Casadevall (1991) to have mature gonads was 166 mm TL while over 176 mm TL all the males investigated were mature. However, the rocker bone can be present in males of 130 mm TL (Casadevall et al., 1996) and a female of 136 mm TL was found to be fully mature (Casadevall, 1991).

Sound variables were compared to fish TL and morphology. The five males were measured with a caliper square and radiographed under 43 kV and 10 mAs⁻¹ with a DigiVeX FP (MEDEX Loncin S.A., Belgium) on June 1, 2011, July 5, 2011, and July 29, 2011 at the Veterinary institute in University of Liège. Maximal height and length of the swimbladder, maximal length of the gaseous part of the swimbladder [previous observations on dissected fish indicated that the anterior swimbladder fills partially with a gelatinous substance in mature males (see: Casadevall et al., 1996)], internal tube length, and rocker bone diameter were measured in Adobe Photoshop (Adobe, San Jose, CA) and pixels were converted to mm based on fish TLs. Morphological structures investigated are illustrated in a schematic manner in Figure 3 to facilitate understanding.

All experimental procedures were approved by the University of Liège Institutional Animal Care and Use Committee.

Statistical Tests

Principal component analysis (PCA) was performed with the sounds of the five males. This analysis determined differences between male sounds based on an overview of sound distribution in a plane defined by the first two principal components. Data from eight sound characteristics were utilized to compute these principal components. The scatter plot illustrated the weight of each sound variable in these components. Linear regressions using log-transformed data were done to investigate the variations in swimbladder structures and sounds between the five males. The slopes were presented in degrees (arctan of the slopes) to facilitate comparisons. These tests were done in Statistica 10 (Statsoft, Tulsa, OK).

RESULTS

Sound Characteristics

Differences between the five male sounds were first explored with a PCA using eight variables (Fig. 4a): PN, PD, AS, A, SP, LP, first peak frequency (F1), and second peak frequency (F2).

The first three principal components explained almost 80% of the total variation (Table 2). PC1 (47.6%) and PC2 (18.8%) alone explained more than 66% of the total variance (Table 2; Fig. 4a). PC1 was loaded mainly by PD, AS, SP, and first and second peak frequencies (Table 2; Fig. 4a). The relative importance of A and LP was lower but not negligible while PN had almost no effects on PC1. PC2 was mainly loaded by PN, SP, and LP (Table 2; Fig. 4a). The biplot of sounds from each fish along the first two principal components (PC1 and PC2) showed overlap among individuals, except for sounds produced by male 1 which was also the smallest specimen (Fig. 4a). Male 1 mainly differed from the other males along PC1. Thus, the variables with high loads on PC1 should also present important differences between male 1 and the other males. Means and standard deviations for the five variables that mainly affected PC1 are illustrated in Figure 5. Sounds

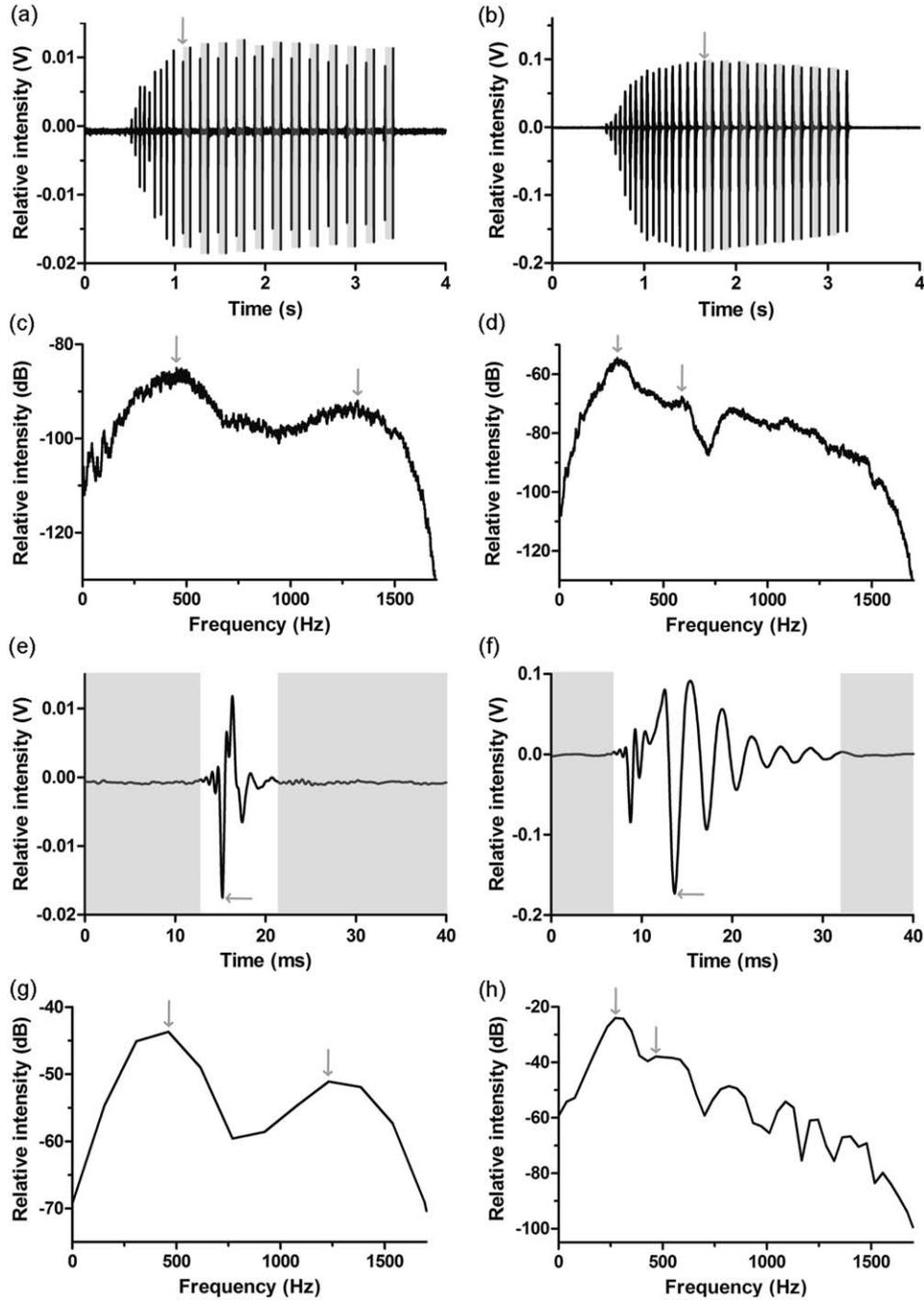


Fig. 2. *Ophidion rochei*, multiple-pulsed sounds produced by two males with differences in their sonic apparatuses. Waveforms (**a**, **b**) and power spectra (**c**, **d**) of multiple-pulsed sounds produced by male 1 (**a**, **c**) and male 5 (**b**, **d**). Power spectra performed on complete sounds are smoothed (average over 31 points). Waveform (**e**) and power spectrum (**g**) from one pulse isolated from multiple-pulsed sounds produced by male 1. Waveform (**f**) and power spectrum (**h**) from one pulse isolated from multiple-pulsed sounds produced by male 5. Grey arrows show the ASs (**a**, **b**), the amplitude maxima (**e**, **f**), or the first and second peak frequency (**c**, **d**, **g**, **h**). In (**a**) and (**b**), grey areas correspond to the short pulse periods (Long pulse periods are the white areas between two successive short periods). PD is shown in (**e**) and (**f**) by the white area.

from male 1 had the highest peak frequencies, earliest AS, and shortest PD and SP (Fig. 5). Although A had a lower loading on PC1, mean amplitude varied greatly and was much lower in male 1 (Table 1).

Sounds produced by male 1 formed a well isolated cluster in the biplot of PC1 and PC2 (Fig. 4a) and the distinctiveness of male 1 may obscure differences among the other fish. A second PCA (Table 3; Fig. 4b), conducted with all fish except

TABLE 1. *Ophidion rochei*, comparison between 5 males using morphological data and sound characteristics

		Morphological data					r^2	Slope (degrees)
		Male 1	Male 2	Male 3	Male 4	Male 5		
TL (mm)	Mean	133	153.3	166.8	169.3	169	1	45°
	SD	0	0.6	0.8	1.2	0		
SB length (mm)	Mean	20.0	24.3	27.8	28.1	28.6	1	56°
	SD	0.6	1.2	0.6	1.1	0.3		
SB height (mm)	Mean	6.4	8.0	9.0	9.1	9.1	1	56°
	SD	0.5	0.5	0.3	0.5	0.3		
GB length (mm)	Mean	19.1	22.2	22.8	23.8	23.2	0.96	39°
	SD	0.5	1.3	1.7	2.2	2.5		
Tube length (mm)	Mean	1.5	4.1	6.1	5.7	5.0	0.95	79°
	SD	0.3	1.7	0.9	1.5	0.5		
RB diameter (mm)	Mean	1.6	3.4	4.5	4.1	4.2	0.95	76°
	SD	0.3	0.4	0.1	0.4	0.1		
		Sound characteristics					r^2	Slope (degrees)
		Male 1	Male 2	Male 3	Male 4	Male 5		
Pulse duration (ms)	Mean	7.5	10.9	10.4	12.9	15.3	0.77	66°
	SD	2.8	2.6	1.4	2.9	1.7		
Alternation St.	Mean	12.3	15.7	17.2	16.2	17.5	0.92	53°
	SD	1.4	2.1	1.1	1.9	1.8		
first peak fr. (Hz)	Mean	454	244	206	187	266	0.8	-71°
	SD	33	55	26	21	36		
second peak fr. (Hz)	Mean	1579	657	466	402	498	0.97	-79°
	SD	81	316	133	14	93		
Short period (ms)	Mean	82.9	95.6	94.3	94.9	91	0.63	24° (NS)
	SD	2.8	0.9	0.7	2.3	1.6		
Long period (ms)	Mean	124.6	122.8	109.4	123.7	104.5	0.35	-25° (NS)
	SD	17.7	2.2	1.2	2.8	1.9		
Pulse number	Mean	34.5	28.3	31.7	36.6	32.3	0	0.5° (NS)
	SD	4.6	4	1.3	4.7	3.4		
re_Amp. (mV)	Mean	7	27	49	20	64	0.71	82° (NS)
	SD	3	15	12	7	46		

Mean values and standard deviations of morphometric data (measured on the three radiographs realized for each fish) and sound characteristics. Values in degrees correspond to the arctangent of the slopes obtain from the linear regression of the log-transformed variables ($Y = \log(\text{variable})$ and $X = \log(\text{TL})$). The determination coefficient r^2 is given for each variable compared to total length. First peak fr.: first peak frequency. Second peak fr.: second peak frequency. Alternation St.: alternation start. re_Amp.: relative pulse amplitude. GB: gas bubble (proportion of swimbladder that seems filled with gas = SB without gelatinous substance). NS: slope did not differ significantly ($p < 0.05$) from zero. Pulse number: number of pulses in a call. RB: rocker bone. SB: swimbladder. TL: total length. Tube: internal tube.

male 1, suggested that they were also differences between the other males: especially between male 2, male 5, and the two other males (Fig. 4b). All together, these observations suggest that male 1 produced the most atypical sounds of the five males and that, among the remaining males (2–5), male 2 sounds seemed the most similar to male 1 sounds (Figs. 4, 5). The waveforms and power spectra shown in Figure 2 illustrate the differences in sounds produced by male 1 and male 5.

The sound characteristic means shown in Table 1 were log-transformed and plotted against log-transformed fish TL. Equations of linear regressions were calculated and the arctangent of each slope gave an angle in degrees for each regression (Table 1). PD, A, and first and second peak fre-

quency, varied strikingly over a small TL range. For PD, A, and first and second peak frequency the angle of the slope exceeded 65° in absolute value. Note the angle was positive for PD and A while it was negative for both peak frequencies (Table 1). Averaged sound amplitude in male 1 was at least 9 dB lower than in the other males (18 dB lower than in male 3 and 5). However, this variable must be treated with caution because the sound amplitude can vary on short distances. In addition, despite a coefficient of determination of 0.71 and a slope of 82°, linear regression of A on TL was not significant (Table 1). The angle of the linear regression reached 53° for AS while it was smaller than 30° for the nonsignificant linear regressions of the remaining sound variables (Table 1).

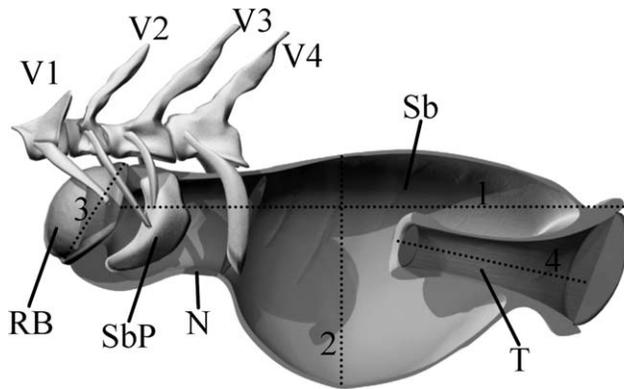


Fig. 3. *Ophidion rochei*, identification of the structures related to the swimbladder in males. Left lateral view of the bones of the sonic apparatus (first four vertebrae and epineurals) and medial view of the right half of the swimbladder (left side of the swimbladder has been removed). N: swimbladder neck. RB: rocker bone. Sb: swimbladder. SbP: swimbladder plate. T: internal tube. V1–4: vertebra 1–4. Dotted lines: (1) maximal swimbladder length, (2) maximal swimbladder height, (3) maximal rocker bone diameter, and (4) internal tube length.

Size and Morphology of Isolated Males

Little to no measurable growth in TL occurred in captive males from the period of early June to late July: 133 mm for male #1 (no growth), 153 to 154 mm for male #2, 166 to 167.5 mm for male #3, 168 mm to 170 mm for male #4, and 169 mm for male #5 (no growth). The TL of the smallest male (#1) was about 80% of the largest one (#4).

Differences in swimbladder morphology were revealed by radiographs (Fig. 6). Fish length was highly correlated ($r^2 \geq 0.92$) with swimbladder height and length, indicating that longer fish had longer and larger swimbladders (Table 1). Thus, measurements on radiographs showed no clear

increase in swimbladder length or height in any males except in male 2 where swimbladder length and height increased 2.5 mm and 1 mm, respectively.

Males 2, 3, 4, and 5 had similar morphologies. However, the rocker bone of males 2 and 4 appeared slightly less developed (or mineralized) in comparison with males 3 and 5 (Fig. 6). During the first part of the recording period, the internal tube of male 2 was also less developed than in male 3, 4, and 5. The anterior swimbladder of male 3 appeared filled by a gelatinous substance (Fig. 6). This substance was not observed in early June in swimbladders of male 2, 4, and 5 but appeared progressively. Although the gelatinous substance was not present in the swimbladder of male 1, the maximal length of its “gas bubble” was shorter than those of the remaining males (Table 1). For all swimbladder structures investigated, male 1 had the most striking differences: least developed rocker bone and internal tube, and no gelatinous substance in the swimbladder (Fig. 6). Moreover, the obvious neck in the anterior part of other male swimbladders was not clearly visible in male 1.

Morphometric data from Table 1 were log-transformed to investigate allometries suggested by observations on Figure 6. Each variable was compared to the TL ($r^2 \geq 0.95$) and, except for gas bubble length (slope < 1), positive allometries (slope > 1) were found (Fig. 7). Allometries were relatively moderate for swimbladder height, swimbladder length, and gas bubble length but much more pronounced for rocker bone diameter and internal tube length (Fig. 7). The regression line for rocker bone diameter and internal tube had an angle of 76° and 79° , respectively, while its was

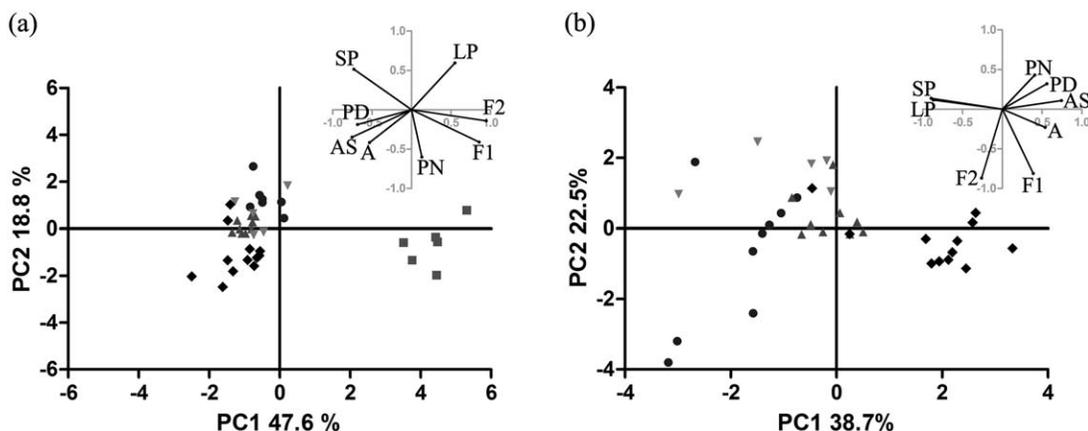


Fig. 4. *Ophidion rochei*, scatterplot of principal component analyses (PCAs) performed on eight variables from multiple pulsed-sounds of males. Sounds were recorded from male 1 (squares), 2 (circles), 3 (triangles), 4 (inverted triangles), and 5 (diamonds), that were successively placed in a tank. The two PCAs (a and b) displayed were based on sounds recorded at 21.5°C . The PCA represented in (b) was run without male 1. The weight of each variable in PC1 and PC2 is illustrated on the factorial subplot in the right upper side of PCAs. The eight sound variables analyzed in the PCAs were: pulse amplitude (A), pulse number (PN), pulse duration (PD), alternation start (AS), short pulse period (SP), long pulse period (LP), first peak frequency (F1), and second peak frequency (F2).

TABLE 2. *Ophidion rochei*, eigenvalues and factor loadings for the first three axes of the PCA performed on the five males

	PC 1	PC 2	PC 3
Eigenvalues	3.81	1.50	0.96
% of total variance	47.62	18.78	11.96
Cumulative % of total variance	47.62	66.4	78.36
Factor loadings			
first peak fr.	0.86	-0.41	0.17
second peak fr.	0.95	-0.14	0.15
Alternation st.	-0.75	-0.35	-0.22
Pulse number	0.13	-0.6	-0.75
Pulse duration	-0.68	-0.19	0.10
re_Amp.	-0.53	-0.42	0.45
Short period	-0.73	0.52	-0.14
Long period	0.55	0.6	-0.26

First peak fr.: first peak frequency. Second peak fr.: second peak frequency. Alternation St.: alternation start. re_Amp.: relative pulse amplitude

equal or lower than 56° for the other variables (Table 1). In the studied sample, the two former structures grew much faster than the fish. Conversely, the length of the gas bubble grew more slowly than the fish. The latter observation is consistent with the hypothesis that swimbladder was progressively filled with a gelatinous substance.

DISCUSSION

Interindividual Differences

Relationships between body size and sound characteristics have been highlighted in many fish species (Ladich et al., 1992; Myrberg et al., 1993; Lobel and Mann, 1995; Pruzsinszky and Ladich, 1998; Connaughton et al., 2000; Amorim et al., 2003; Colley et al., 2009; Lechner et al., 2010; Tellechea et al., 2010; Boyle and Tricas, 2011; Tellechea et al., 2011; Bertucci et al., 2012; Kéver et al., 2012). Typically, sound frequencies vary inversely with body size within a species, while size-related modifications at the level of the mechanism have been generally ignored. However, because there are important differences in body shape between fish species, similar increases in fish length or mass probably cause different changes in structures involved in sound production.

In this study, differences in some sound characteristics were also seen between the smallest male (#1) and the others. Male #1 displayed the highest peak frequencies, the earliest AS, the lowest A, and the shortest PD and SP (Table 1). However, the differences between the sound characteristics are more pronounced than expected on the basis of the fish size. This means that other characteristics besides fish size should affect sound features.

Morphology of the sonic mechanism seems better suited than size to explain differences between the specimens. Male #1 had a less developed rocker bone, internal tube and neck, and a swim-

TABLE 3. *Ophidion rochei*, eigenvalues and factor loadings for the first three axes of the PCA performed on four males (male 1 was excluded)

	PC 1	PC 2	PC 3
Eigenvalues	3.09	1.80	1.10
% of total variance	38.68	22.49	13.71
Cumulative % of total variance	38.68	61.17	74.88
Factor loadings			
first peak fr.	0.39	-0.81	0.16
second peak fr.	-0.26	-0.87	0.20
Alternation st.	0.74	0.11	0.48
Pulse number	0.41	0.43	0.71
Pulse duration	0.56	0.32	-0.45
re_Amp.	0.54	-0.23	-0.18
Short period	-0.89	0.14	0.12
Long period	-0.87	0.12	0.23

First peak fr.: first peak frequency. Second peak fr.: second peak frequency. Alternation St.: alternation start. re_Amp.: relative pulse amplitude

bladder devoid of gelatinous substance; acoustic structures in male #2 were also slightly less developed than in male #3, 4, and 5 (Table 1 and Fig. 6). The positive allometric growth of rocker bone diameter and internal tube length were high, the slopes exceeding 75° . Similar slope angles were found for PD, peak frequencies, and A (Table 1).

Ramcharitar et al. (2006) compared weakfish and spotted seatrout sounds and suggested that some differences in sound characteristics could be correlated with differences in swimbladder morphology while Parmentier et al. (2003) and Colley et al. (2012) showed that sound characteristics are affected by modifications in skeletal elements like epineurals and swimbladder plates. Peak frequency, PD, and A are likely affected by the appearance and growth of the gelatinous substance, internal tube, and rocker bone because these modifications most probably affect the vibration of the swimbladder, epineurals, and swimbladder plates. A highly variable LP was only found in male 1 (Table 1), which suggests that its sonic muscles had not reached their maximal efficiency. Finally, the fact that PN was not affected by the changes in size and morphology hint that this sound characteristic is fixed at a neuronal level.

In Batrachoididae, sexual dimorphism in sonic muscles is thought to explain some of the differences between male and female sound characteristics (Fine et al., 1990; Brantley et al., 1993). Kéver et al. (2012) observed that sonic muscles were present in any *O. rochei* but appeared proportionally larger in males. It results positive allometric growth of sonic muscles during male maturation may also partly explain some sound differences observed between individuals in this study. However, such information cannot be extracted from radiographs and fish could not be dissected for comparisons. Swimbladder plates, despite being

mineralized, were too small to be seen on radiographs but also should undergo substantial modification during male puberty (Kéver et al., 2012).

General Considerations

An important finding from this study lies in the marked differences between the sonic apparatus morphology (and resulting calls) of different males. This also parallels previous results from Kéver et al. (2012) that highlighted substantial differences in sonic apparatus morphology and sounds of male and female *O. rochei*. It seems unlikely that *O. rochei* uses vision to distinguish conspecifics and to assess external male secondary sexual characteristics. This species, with a nocturnal mode of life, has probably invested in acoustic characters for distinguishing individuals and sexes. In many other prolific callers, such as pomacentrids and cichlids, sounds are generally used in conjunction with visual signals (Mann and Lobel, 1997; Mann and Lobel, 1998; Parmentier et al., 2009; Bertucci et al., 2010; Parmentier et al., 2010b; Longrie et al., 2013): the sounds reinforce the behavior, but the visual signal can occur without the production of sounds. In these species the assessment of caller fitness and characteristics do not rely only on sounds. Consequently, natural selection acting on the sonic apparatus is probably weaker than in *Ophidion rochei*. The long duration, intense, pulsatile calls produced by males should facilitate listening fish in the location of sound producers. Female sounds, however, are much shorter and appear less conspicuous. Moreover, calls of mature males (with a fully developed sonic apparatus) should be easier to detect than calls of males that do not have a fully developed rocker bone, internal tube, and so forth. Mature males produce lower frequency calls composed of much longer, more intense pulses. The advantage of the latter two features is evident. It is less intuitive to assess the advantages conferred by modifications in call frequencies because background noise characteristics, sound propagation in the natural environment, and hearing capabilities of the receiver must be considered. In shallow water, very low frequencies do not propagate (Fine and Lenhardt, 1983; Rogers and Cox, 1988; Mann, 2006), while snapping shrimp generally dominate sea biotic noise over 2 kHz (Cato and Bell, 1992; Cato, 2008; Radford et al., 2008; Kennedy et al., 2010). Although the hearing capabilities of *O. rochei* are unknown, fish with no accessory auditory structures that enhance hearing acuity generally hear best below 1 kHz (Popper and Fay, 2011; Ladich and Fay, 2013). Thus, the sonic apparatus of mature males

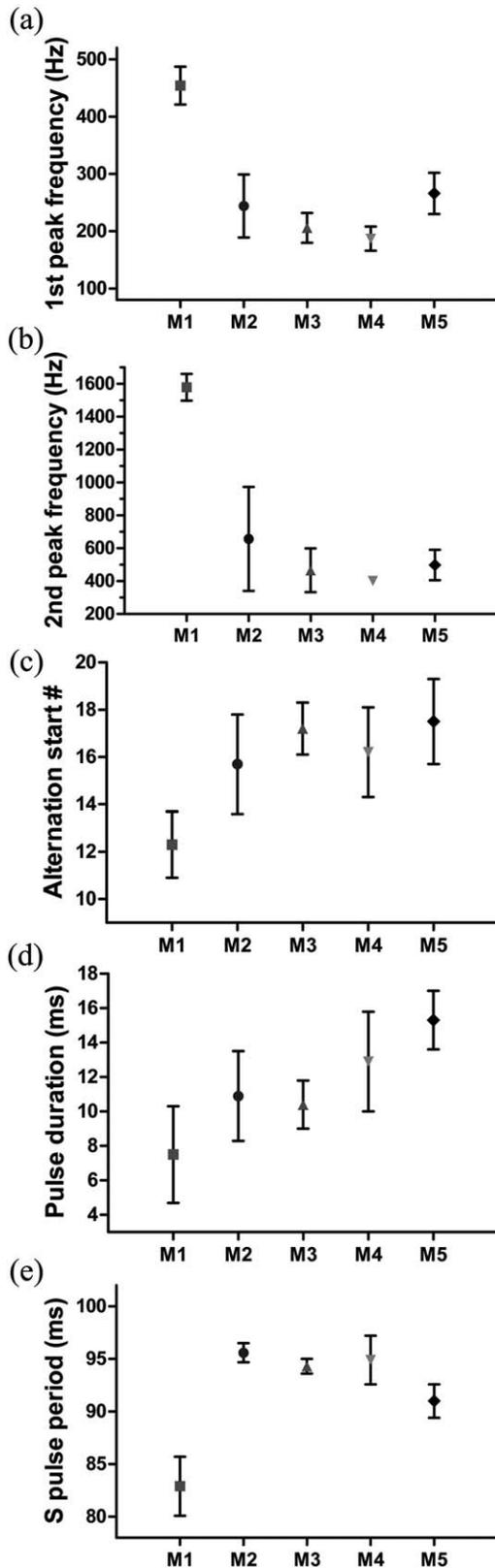


Fig. 5. *Ophidion rochei*, mean values and standard deviations of five variables from the sounds of five males. Mean values and standard deviations for (a) first peak frequency, (b) second peak frequency, (c) alternation start, (d) pulse duration, and (e) short pulse period. Mean values and standard deviations were calculated from sounds recorded at 21.5°C for each male: male 1 (M1), 2 (M2), 3 (M3), 4 (M4), and 5 (M5).

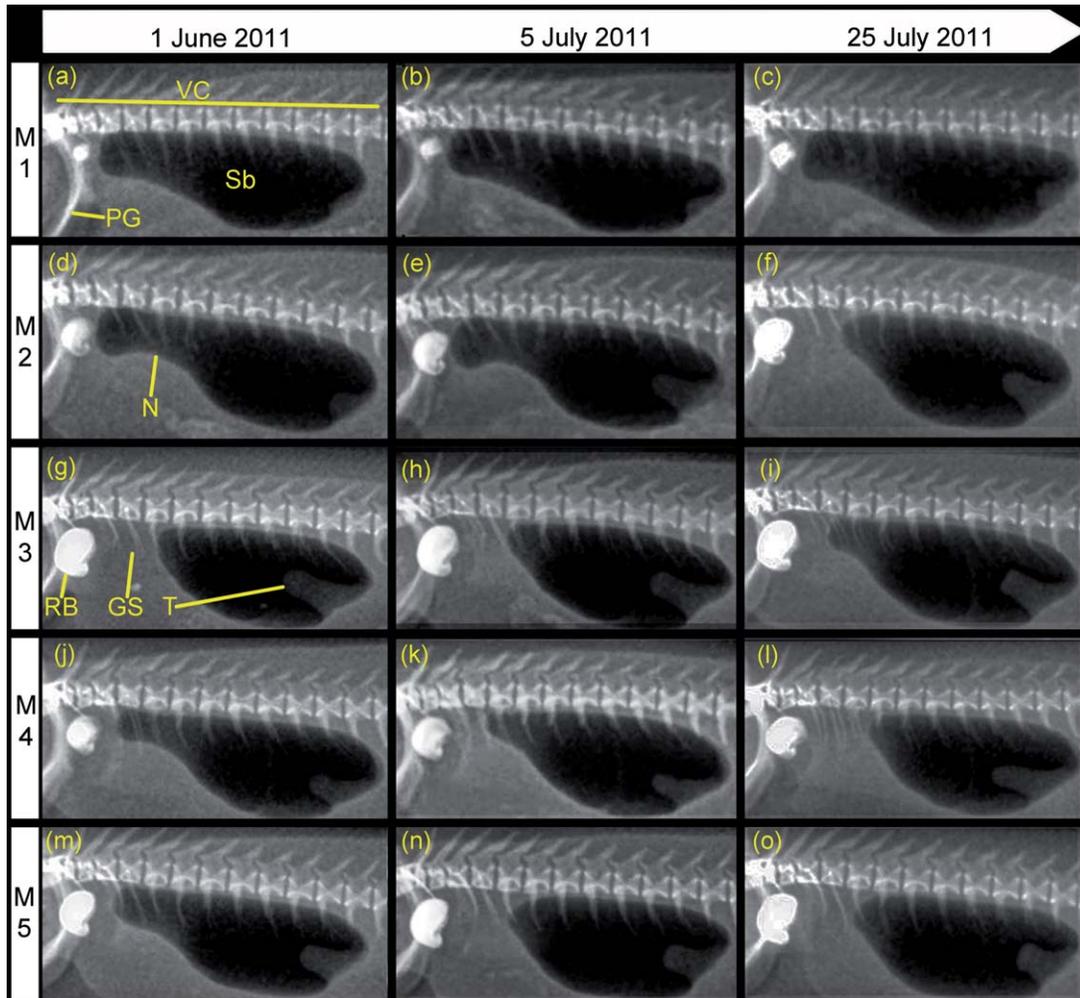


Fig. 6. *Ophidion rochei*, radiographs of swimbladder areas in 5 males taken over the study period. Males 1 (a, b, c), 2 (d, e, f), 3 (g, h, i), 4 (j, k, l), and 5 (m, n, o) were radiographed the first of June 2011 (a, d, g, j, m), the fifth of July (b, e, h, k, n), and the 25th of July (c, f, i, l, o). Each picture displayed a left lateral view of the anterior part of the vertebral column (VC), the posterior part of the pectoral girdle (PG), and the swimbladder (Sb). The swimbladder showed different stages of development in swimbladder structures: rocker bone (RB), anterior neck (N), and internal tube (T). Some photographs presented a swimbladder partially filled with a gelatinous substance (GS). M1–5: male 1–5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

probably evolved to produce sounds with the best compromise between effective propagation in a marine environment, reducing masking from background noise, and matching conspecific hearing abilities.

Ophidion rochei and *O. barbatum* have similar external phenotypes but differ in many respects, especially in terms of the rocker bone, swimbladder plate, and swimbladder shape (Casadevall et al., 1996; Parmentier et al., 2006a; Parmentier et al., 2010a; K  ver et al., 2012). Regrettably, *O. barbatum* sounds were never recorded. Differences in internal morphology should be important for most Ophidiidae species since many of them are active in the dark, are devoid of external secondary sexual characters, and have a species-specific sonic apparatus (see for example: Rose, 1961;

Courtenay, 1971; Carter and Musick, 1985; Casadevall et al., 1996; Mann et al., 1997; Nielsen et al., 1999; Parmentier et al., 2006a; Parmentier et al., 2010a; K  ver et al., 2012). Many ophidiid sonic apparatuses investigated so far showed striking differences in swimbladder shape and composition, in sonic muscles, and in modified bones (see: Casadevall et al., 1996; Courtenay, 1971; Fine et al., 2007; Parmentier et al., 2006a; Rose, 1961).

The important ontogenetic changes to the sonic apparatus during maturation of *O. rochei* (K  ver et al., 2012) and their effects on sound characteristics, as well as the high diversity in ophidiid sonic apparatus morphologies, suggest that acoustic communication is a key factor for evolutionary success and speciation in this taxon.

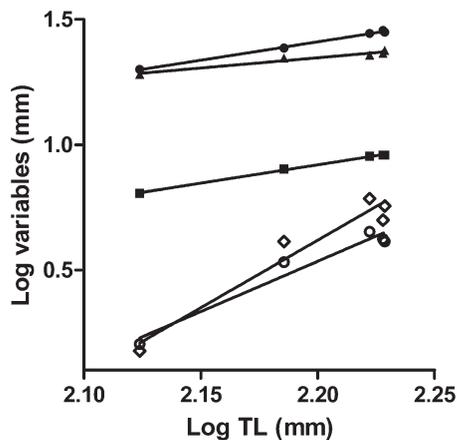


Fig. 7. *Ophidion rochei*, plot of the log-transformed morphometric variables measured on five males. Circles: maximal swimbladder length (SBI). Squares: maximal swimbladder height (SBh). Triangles: maximal gas bubble length (GB). Empty diamonds: internal tube length (T). Empty circles: rocker bone diameter (RB). Equations of the linear regressions: $y = 1.46x - 1.80$ (SBI), $y = 1.47x - 2.31$ (SBh), $y = 0.82x - 0.54$ (GB), $y = 5.39x - 11.24$ (IT), and $y = 4.02x - 8.31$ (RB). All the slopes significantly ($P < 0.05$) differ from 0. TL: total length.

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